FILE 'CAPLUS' ENTERED AT 11:04:20 ON 22 SEP 2004

=> S RAT GENE;S REGULATOR(3A)AUTOLYSIS
        660519 RAT
        631802 RATS
       1040190 RAT
                (RAT OR RATS)
        887156 GENE
        335099 GENES
        939059 GENE
                (GENE OR GENES)
L1        2460 RAT GENE
                (RAT(W)GENE)

         60788 REGULATOR
         80220 REGULATORS
        126344 REGULATOR
                (REGULATOR OR REGULATORS)
          6902 AUTOLYSIS
L2           9 REGULATOR(3A)AUTOLYSIS

=> D 1-9 CBIB ABS

L2 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
2003:494561 Document No. 139:256009 Characterization of RAT, an **autolysis regulator** in Staphylococcus aureus. Ingavale, S. S.; Van Wame, W.; Cheung, A. L. (Department of Microbiology, Dartmouth Medical School, Hanover, NH, 03755, USA). Molecular Microbiology, 48(6), 1451-1466 (English) 2003. CODEN: MOMIEE. ISSN: 0950-382X. Publisher: Blackwell Publishing Ltd..

AB In trying to identify genetic loci involved in the regulation of cap5 genes in Staphylococcus aureus, we isolated a transposon mutant that exhibited a growth defect, enhanced autolysis and increased sensitivity to Triton X-100 and penicillin, attributable in part to increased murein hydrolase activity. Anal. of the chromosomal sequence flanking the transposon insertion site revealed that the gene disrupted in the mutant encodes an open reading frame of 147 amino acids. We named this gene rat, which stands for regulator of autolytic activity. Sequence anal. indicated that Rat is homologous to the MarR and, to a lesser extent, the SarA protein families. Mutations in rat resulted in decreased expression of known autolytic regulators lytSR, lrgAB and arlRS. Gel shift studies indicated that Rat binds to the lytRS and arlRS promoters, thus confirming Rat as a DNA-binding protein to these known repressors of autolytic activity. As anticipated, rat appears to be a neg. regulator of autolysin genes including lytM and lytN. These data suggest that the rat gene product is an important regulator of autolytic activity in S. aureus.

L2 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1998:463375 Document No. 129:186580 Opposing roles of the Staphylococcus aureus virulence regulators, Agr and Sar, in Triton X-100- and penicillin-induced autolysis. Fujimoto, David F.; Bayles, Kenneth W. (Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID, 83844-3052, USA). Journal of Bacteriology, 180(14), 3724-3726 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The regulation of murein hydrolases is a critical aspect of peptidoglycan growth and metabolism In the present study, we demonstrate that mutations within the Staphylococcus aureus virulence factor regulatory genes, agr and

sar, affect autolysis, resulting in decreased and increased autolysis rates, resp. Zymog. analyses of these mutant strains suggest that agr and sar exert their effects on autolysis, in part, by modulating murein hydrolase expression and/or activity.

L2 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1994:187280 Document No. 120:187280 Cell-wall autohydrolysis in isolated endosperms of lettuce (Lactuca sativa L.). Dutta, Sunil; Bradford, Kent J.; Nevins, Donald J. (Dep. Veg. Crops, Univ. California, Davis, CA, 95616-8741, USA). Plant Physiology, 104(2), 623-8 (English) 1994. CODEN: PLPHAY. ISSN: 0032-0889.

AB Cell walls prepared from the endosperm tissue of hydrated lettuce (Lactuca sativa L.) seeds undergo autohydrolysis. Release of carbohydrates is most rapid (0.4-0.6 μg per endosperm) within the 1st h of incubation in buffer, but substantial autolysis is sustained for at least 10 h. Autolysis is temperature sensitive, and the optimum rate occurs at pH 5. The rate of autolysis increases markedly in the period just prior to radicle emergence. The cell-wall polysaccharide composition in micropylar and lateral endosperm regions differs significantly; the micropylar walls are rich in arabinose and glucose with substantially lower amts. of mannose. Although walls prepared from both micropylar and lateral regions undergo autolysis, micropylar walls release carbohydrates at a higher rate than lateral walls. Autolysis products elute as large polymers when subjected to size-exclusion chromatog., suggesting that endo-enzyme activity is responsible for release of fragments containing arabinose, galactose, mannose, and uronic acids. Arabinose, galactose, mannose, and glucose are also released as monomers. As a function of time, the ratio of polymers to monomers decreases, indicating that exo-enzyme activity is also present. Thermoinhibition or treatment with abscisic acid suppresses germination and reduces the rates of autolysis of walls isolated from the endosperm by .apprx.25%. Treatments that alleviate thermoinhibition (kinetin and gibberellic acid) increase the rates of autolysis by 20 to 30% when compared to thermoinhibited controls.

L2 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1990:214035 Document No. 112:214035 Effect of auxin on autolysis of cell walls in adzuki bean epicotyls. Hoson, Takayuki (Fac. Sci., Osaka City Univ., Osaka, 558, Japan). Plant and Cell Physiology, 31(2), 281-7 (English) 1990. CODEN: PCPHA5. ISSN: 0032-0781.

AB Native cell walls of adzuki bean (Vigna angularis) epicotyls incubated in buffer autolytically released neutral sugars, abundant in galactose and uronic acids. Treatment with 10-5 M IAA of subapical or basal epicotyl segments for 3 h did not influence the amount of total neutral sugars released from the cell walls during autolysis. However, the amount of glucose and xylose released from subapical cell walls was increased by IAA. Pretreatment with IAA of subapical epicotyl segments enhanced the solubilization of neutral sugars from pectinase-treated cell walls during incubation in buffer at pH 5 to 6. The amount of fucose, xylose, and glucose released was specifically increased by IAA. Of the sugar fractions released from pectinase-treated cell walls during autolysis and subsequently separated by gel filtration on a Toyopearl HW-40S column, IAA promoted the release of oligosaccharides, consisting mainly of glucose and xylose. These results suggest that autolytic degradation of xyloglucans is closely related to IAA-induced growth of adzuki bean epicotyls.

L2 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1986:145383 Document No. 104:145383 Antibiotics and polyelectrolytes modulate bacteriolysis and the capacity of bacteria to trigger an oxygen

burst in neutrophils. Ginsburg, Isaac; Borinski, Ruth; Sadovnik, Milu; Shauli, Sara; Wecke, J.; Giesbrecht, P.; Lahav, Meir (Hadassah Fac. Dent. Med., Hebrew Univ., Jerusalem, Israel). Influence Antibiot. Host-Parasite Relat., [Proc. - Int. Symp.], 2nd, 141-51. Editor(s): Adam, Dieter; Hahn, Helmut; Opferkuch, Wolfgang. Springer: Berlin, Fed. Rep. Ger. (English) 1985. CODEN: 54XQAI.

AB The biodegrdn. of bacteria by leukocytes in infectious and inflammatory foci may involve direct enzymic cleavage by lysosomal hydrolases as well as the activation by leukocyte and tissue-derived cationic proteins and phospholipases of the bacterial autolytic wall enzymes. Antibiotics of the lactam series may facilitate **autolysis** by removing **regulators** of the autolytic wall enzymes (i.e. acid phospholipids) thus leading to degradation of the bacterial cell walls from within. However, a variety of sulfated polyelectrolytes (dextran sulfate, heparin, polyanethole sulfonate) markedly inhibited penicillin G lysis of Staphylococcus aureus, presumably due to their capacity to inhibit autolytic wall enzymes. Staphylococci grown in the presence of subinhibitory concns. of penicillin G became much more susceptible to lysis by leukocyte exts. and by lysozyme as compared to controls. This process was also markedly inhibited by anionic polyelectrolytes, which presumably interfered with the activity of the bacterial autolysines. Both intact staphylococci and penicillin-grown cells which had been injected intraarticularly into the knee joints of rats underwent massive plasmolysis within macrophages, but no apparent degradation of their cell walls was evident by electron microscopy, suggesting that the accumulation of inflammatory exudates, rich in anionic polyelectrolytes, might have been responsible for this effect. Staphylococci and group A streptococci, which had been cultivated in the presence of subinhibitory concns. of penicillin G or cephalosporins induced a much lesser generation of chemiluminescence and superoxide responses in human PMNs when a variety of cationic and anionic agents were employed as ligands upon such bacteria. The results suggest that the antibiotics modulated bacterial surfaces. The survival of undegraded microbial cell wall components in tissue, even under penicillin treatment, may explain the perpetuation and propagation of certain post infectious sequellae. Bacteria treated with antibiotics may also generate lesser amts. of toxic O radicals, which may contribute to longer survival of viable microorganisms in the tissues of the host. The phlogistic role played by degradation products of bacteria is discussed.

L2 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1985:59002 Document No. 102:59002 Composition of agents regulating bacterial autolysis. Bandoyan, A. K.; Kislukhina, O. V.; Kalunyants, K. A. (Vses. Nauchno-Issled. Biotekh. Inst., Moscow, USSR). Mikrobiologiya, 53(6), 942-6 (Russian) 1984. CODEN: MIKBA5. ISSN: 0026-3656.

AB The agents regulating bacterial autolysis were isolated from the lysate of Bacillus subtilis 402, B. subtilis R2, and Micrococcus lysodeikticus by extraction with 5% TCA followed by precipitation with 5 vols. of isopropranol. Fractions activating bacterial autolysis and fractions inhibiting it were found in all of the prepns. after separation on Acrylex P-60. Fractions with a mol. mass <12,000 daltons activated the autolysis whereas fraction with a mol. mass >18,400 daltons inhibited it. The activity of fractions inhibiting autolysis decreased while that of fractions activating autolysis increased in the regulating agents isolated from B. subtilis cultures with cell aging. The ability to activate autolysis correlated with the content of amino groups and phosphate, whereas the capacity to inhibit autolysis correlated with the content of reducing sugars in the fractions. The prepns. of the fraction which activated the autolysis from B. subtilis R2 contained 18 amino acids with the predominance of alanine, glutamic acid, lysine, and phenylalanine. Apparently, the regulating properties of the prepns. are promoted by teichoic acids and associated peptidoglycan and protein fragments.

L2 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1982:177810 Document No. 96:177810 Effect of **regulators** on bacterial **autolysis**. Kislukhina, O. V.; Kuznetsova, T. A.; Aksenovskaya, V. E. (Vses. Nauchno-Issled. Biotekh. Inst., Moscow, USSR). Mikrobiologiya, 51(1), 85-9 (Russian) 1982. CODEN: MIKBA5. ISSN: 0026-3656.

AB The teichoic acid (TA) fraction isolated from 23 microorganisms (2 fungi and 21 bacteria) acted as a bioregulator of autolysis in Bacillus subtilis, Escherichia coli, Streptococcus lactis, and to a lesser extent in B. megaterium, B. mesentericus, and B. amyloliquefaciens. TA either activated or inhibited autolysis, depending on the concentration used, the bacterial species, and the age of the culture. TA may be used in various microbiol. processes, such as production of lytic enzymes, preparation of biomass hydrolyzates and autolyzates, and production of high yields of single-cell proteins.

L2 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1981:440807 Document No. 95:40807 Biosynthesis of lytic enzymes in Bacillus subtilis cultures. Kislukhina, O. V.; Kalunyants, K. A.; Markaryan, B. A.; Kuznetsova, T. A. (Vses. Nauchno-Issled. Inst. Biotekh., Moscow, USSR). Biologicheskii Zhurnal Armenii, 34(3), 240-6 (Russian) 1981. CODEN: BZARAZ. ISSN: 0366-5119.

AB The synthesis of lytic enzymes by B. subtilis 402 grown in a mineral medium was enhanced by dilution of the culture with H20 or a salt solution Dilution of the culture 1.75-2.5-fold increased the yield of lytic enzymes by 60-70%. Activation of lytic enzyme biosynthesis was also achieved by addition of **autolysis regulators** which minimized the intensity of autolysis of the bacteria. Teichoic acid [9041-38-7] from the cell wall of various bacteria was used as an effective **regulator** of **autolysis**.

L2 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1979:553950 Document No. 91:153950 Lipoteichoic acid from Staphylococcus aureus: regulation of autolysis and killing of penicillin. I. Role of lipoteichoic acid as a **regulator** of **autolysis**.
Shimatani, Masahiro (Dent. Sch., Osaka Univ., Osaka, Japan). Osaka Daigaku Shigaku Zasshi, 24(1), 54-67 (Japanese) 1979. CODEN: ODSZA2. ISSN: 0473-4629.

AB Lipoteichoic acid (LTA) inhibited both autolysis of intact cells and autolysin activity in S. aureus 209P. The chemical deacylated LTA failed to inhibit significantly in the same concentration range. Addition of LTA to the medium of growing S. aureus culture caused some morphol. changes. Apparently, one of the major physiol. functions of LTA may be the control of bacterial autolysin activities.

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=> S MUREIN HYDROLASE;S GLUCOSAMIDASE;S MURAMINIDASE;S SARA OR (SAR(W)A)
           958 MUREIN
           234 MUREINS
          1015 MUREIN
                 (MUREIN OR MUREINS)
         18753 HYDROLASE
          7995 HYDROLASES
         22858 HYDROLASE
                 (HYDROLASE OR HYDROLASES)
L3          135 MUREIN HYDROLASE
                 (MUREIN(W)HYDROLASE)
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```
            40 GLUCOSAMIDASE
             1 GLUCOSAMIDASES
L4           41 GLUCOSAMIDASE
                (GLUCOSAMIDASE OR GLUCOSAMIDASES)


             8 MURAMINIDASE
             1 MURAMINIDASES
L5           9 MURAMINIDASE
                (MURAMINIDASE OR MURAMINIDASES)


           555 SARA
             4 SARAS
           558 SARA
                (SARA OR SARAS)
          7365 SAR
          1349 SARS
          8492 SAR
                (SAR OR SARS)
      18169603 A
            43 SAR(W)A
L6         598 SARA OR (SAR(W)A)

=> S AUTOLYSIS
L7        6902 AUTOLYSIS

=> S L6 AND L7
L8           2 L6 AND L7

=> S L8 NOT L5
L9           2 L8 NOT L5

=> D 1-2 CBIB ABS

L9   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2004 ACS on STN
2004:686273  Identification of sarV (SA2062), a new transcriptional regulator,
     is repressed by SarA and MgrA (SA0641) and involved in the
     regulation of autolysis in Staphylococcus aureus.  Manna, Adhar
     C.; Ingavale, Susham S.; Maloney, MaryBeth; van Wamel, Willem; Cheung,
     Ambrose L. (Department of Microbiology, Dartmouth Medical School, Hanover,
     NH, 03755, USA).  Journal of Bacteriology, 186(16), 5267-5280 (English)
     2004.  CODEN: JOBAAY.  ISSN: 0021-9193.  Publisher: American Society for
     Microbiology.
AB   The expression of genes involved in the pathogenesis of Staphylococcus aureus
     is known to be controlled by global regulatory loci, including agr, sarA, sae,
     arlRS, lytSR, and sarA-like genes.  Here we described a novel transcriptional
     regulator called sarV of the SarA protein family.  The transcription of sarV
     is low or undetectable under in vitro conditions but is significantly
     augmented in sarA and mgrA (norR or rat) (SA0641) mutants.  The sarA and mgrA
     genes act as repressors of sarV expression, as confirmed by transcriptional
     fusion and Northern anal. data.  Purified SarA and MgrA proteins bound
     specifically to sep. regions of the sarV promoter as determined by gel shift
     and DNase I footprinting assays.  The expression of 19 potential target genes
     involved in autolysis and virulence, phenotypes affected by sarA and mgrA, was
     evaluated in an isogenic sarV mutant pair.  Our data indicated that the sarV
     gene product played a role regulating some virulence genes and more genes
     involved in autolysis.  The sarV mutant was more resistant to Triton X-100 and
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penicillin-induced lysis compared to the wild type and the **sarA** mutant, whereas hyperexpression of sarV in the parental strain or the sarV mutant rendered the resultant strain highly susceptible to lysis. Zymog. anal. of murein hydrolase activity revealed that inactivation of the sarV gene results in decreased extracellular murein hydrolase activity compared to that of wild-type S. aureus. We propose that sarV may be part of the common pathway by which mgrA and **sarA** gene products control **autolysis** in S. aureus.

L9 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
2003:494561 Document No. 139:256009 Characterization of RAT, an **autolysis** regulator in Staphylococcus aureus. Ingavale, S. S.; Van Wame, W.; Cheung, A. L. (Department of Microbiology, Dartmouth Medical School, Hanover, NH, 03755, USA). Molecular Microbiology, 48(6), 1451-1466 (English) 2003. CODEN: MOMIEE. ISSN: 0950-382X. Publisher: Blackwell Publishing Ltd..

AB In trying to identify genetic loci involved in the regulation of cap5 genes in Staphylococcus aureus, we isolated a transposon mutant that exhibited a growth defect, enhanced **autolysis** and increased sensitivity to Triton X-100 and penicillin, attributable in part to increased murein hydrolase activity. Anal. of the chromosomal sequence flanking the transposon insertion site revealed that the gene disrupted in the mutant encodes an open reading frame of 147 amino acids. We named this gene rat, which stands for regulator of autolytic activity. Sequence anal. indicated that Rat is homologous to the MarR and, to a lesser extent, the **SarA** protein families. Mutations in rat resulted in decreased expression of known autolytic regulators lytSR, lrgAB and arlRS. Gel shift studies indicated that Rat binds to the lytRS and arlRS promoters, thus confirming Rat as a DNA-binding protein to these known repressors of autolytic activity. As anticipated, rat appears to be a neg. regulator of autolysin genes including lytM and lytN. These data suggest that the rat gene product is an important regulator of autolytic activity in S. aureus.

=> S (L3,L4,L5) AND L7
L10 23 ((L3 OR L4 OR L5)) AND L7

=> S L10 NOT (L5,L9)
L11 20 L10 NOT ((L5 OR L9))

=> D 1-20 CBIB ABS

L11 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2003:928491 Document No. 140:125007 Identification and characterization of a peptidoglycan hydrolase, MurA, of Listeria monocytogenes, a muramidase needed for cell separation. Carroll, Shannon A.; Hain, Torsten; Technow, Ulrike; Darji, Ayub; Pashalidis, Philippos; Joseph, Sam W.; Chakraborty, Trinad (Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, 20742, USA). Journal of Bacteriology, 185(23), 6801-6808 (English) 2003. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB A novel cell wall hydrolase encoded by the murA gene of Listeria monocytogenes is reported here. Mature MurA is a 66-kDa cell surface protein that is recognized by the well-characterized L. monocytogenes-specific monoclonal antibody EM-7G1. MurA displays two characteristic features:. (i) an N-terminal domain with homol. to muramidases from several gram-pos. bacterial species and (ii) four copies of a cell wall-anchoring LysM repeat motif present within its C-terminal domain. Purified recombinant MurA produced in Escherichia coli was confirmed to be an authentic cell wall hydrolase with lytic properties toward cell wall prepns. of Micrococcus lysodeikticus. An

isogenic mutant with a deletion of murA that lacked the 66-kDa cell wall hydrolase grew as long chains during exponential growth. Complementation of the mutant strain by chromosomal reintegration of the wild-type gene restored expression of this **murein hydrolase** activity and cell separation levels to those of the wild-type strain. Studies reported herein suggest that the MurA protein is involved in generalized **autolysis** of L. monocytogenes.

L11 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2003:438598 Document No. 139:242800 Resistance to **autolysis** in vancomycin-selected Staphylococcus aureus isolates precedes vancomycin-intermediate resistance. Boyle-Vavra, Susan; Challapalli, Mamatha; Daum, Robert S. (Department of Pediatrics, The University of Chicago, Chicago, IL, 60637, USA). Antimicrobial Agents and Chemotherapy, 47(6), 2036-2039 (English) 2003. CODEN: AMACCQ. ISSN: 0066-4804. Publisher: American Society for Microbiology.

AB Four clin. U.S. glycopeptide intermediate resistant S. aureus (GISA) isolates were resistant to Triton X-100-induced **autolysis**. Similar resistance was demonstrated in an isolate obtained after a single passage of a susceptible clin. isolate in low-level vancomycin. Strains with the vancomycin-induced Triton X-100 resistance phenotype produced active **murein hydrolases** but were resistant to lysis by **murein hydrolases**.

L11 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2003:413971 Document No. 138:398642 Therapeutic compositions and methods for regulating autolytic processes in bacteria using Staphylococcus aureus gene RAT and truncation mutant. Cheung, Ambrose (USA). U.S. Pat. Appl. Publ. US 2003100002 A1 20030529, 18 pp., Cont.-in-part of U.S. Ser. No. 92,264. (English). CODEN: USXXCO. APPLICATION: US 2002-290142 20021106. PRIORITY: US 2001-PV273791 20010306; US 2001-PV312546 20010815; US 2001-PV329140 20011012; US 2002-92264 20020306.

AB Specifically, disclosed is a Staphylococcus aureus gene RAT, which encodes a 17 kDa protein with 147 residues and plays a role in regulating autolytic activity. In addition, the RAT mutant gene, encoding a protein of 134 residues in length, 13-amino acid C-terminal truncation deletion mutant caused by Tn551 transposon insertion is also provided. More specifically, in the presence of penicillin, the RAT mutant is shown to readily increase lysis as compared to wild-type S. aureus thus enabling the bacteria to survive. Zymog. anal. of cell-associated **murein hydrolases** as well as cell wall morphol. anal. is performed to analyze the autolytic activity of the RAT mutant. Methods for identifying and using agents which interact with the gene or mutant gene or polypeptides encoded thereby to inhibit bacterial growth and infectivity are also provided.

L11 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
2003:296829 Document No. 139:47887 The Staphylococcus aureus cidAB Operon: Evaluation of its role in regulation of **murein hydrolase** activity and penicillin tolerance. Rice, Kelly C.; Firek, Brian A.; Nelson, Jeremy B.; Yang, Soo-Jin; Patton, Toni G.; Bayles, Kenneth W. (Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID, 83844-3052, USA). Journal of Bacteriology, 185(8), 2635-2643 (English) 2003. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB Recent studies have shown that expression of the Staphylococcus aureus lrgAB operon inhibits **murein hydrolase** activity and decreases sensitivity to penicillin-induced killing. It was proposed that the lrgAB gene products function in a manner analogous to an antiholin, inhibiting a putative holin from transporting **murein hydrolases** out of the cell. In the present study the

cidAB operon was identified and characterized based on the similarity of the cidA and cidB gene products to the products of the lrgAB operon. Zymog. and quant. analyses of **murein hydrolase** activity revealed that mutation of the cidA gene results in decreased extracellular **murein hydrolase** activity compared to that of S. aureus RN6390, the parental strain. Complementation of cidA expression restored the wild-type phenotype, indicating that expression of the cidAB operon has a pos. influence on extracellular **murein hydrolase** activity. The cidA mutant also displayed a significant decrease in sensitivity to the killing effects of penicillin. However, complementation of the cidA defect did not restore penicillin sensitivity to wild-type levels. Reverse transcriptase PCR also revealed that cidAB is maximally expressed during early exponential growth, opposite of what was previously observed for lrgAB expression. Based on these results, the authors propose that the cidAB operon encodes the holin-like counterpart of the lrgAB operon and acts in a manner opposite from that of lrgAB by increasing extracellular **murein hydrolase** activity and increasing sensitivity to penicillin-induced killing.

L11 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2002:696099 Document No. 137:227731 Diagnostic and therapeutic compositions and methods for regulating autolytic processes in bacteria using related Streptococcus aureus gene RAT and truncation mutant. Cheung, Ambrose (Trustees of Dartmouth College, USA). PCT Int. Appl. WO 2002070666 A2 20020912, 26 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US6844 20020306. PRIORITY: US 2001-PV273791 20010306; US 2001-PV312546 20010815; US 2001-PV329140 20011012.

AB A nucleic acid sequence required for regulating the autolytic activity of bacteria is provided. Also provided are polypeptides encoded by the gene or mutant gene as well as vector and host cells for expressing these polypeptides. Specifically, disclosed is a Staphylococcus (S.) aureus gene RAT, which encodes a 17 kDa protein with 147 residues and plays a role in regulating autolytic activity. In addition, the RAT mutant gene, encoding a protein of 134 residues in length, 13-amino acid C-terminal truncation deletion mutant caused by Tn551 transposon insertion is also provided. More specifically, in the presence of penicillin, the RAT mutant is shown to readily increase lysis as compared to wild-type S. aureus thus enabling the bacteria to survive. Zymog. anal. of cell-associated **murein hydrolases** as well as cell wall morphol. anal. is performed to analyze the autolytic activity of the RAT mutant. Methods for identifying and using agents which interact with the gene or mutant gene or polypeptides encoded thereby to inhibit bacterial growth and infectivity are also provided.

L11 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2000:670447 Document No. 134:26789 Biological roles of two new **murein hydrolases** of Streptococcus pneumoniae representing examples of module shuffling. Lopez, Rubens; Gonzalez, Maria P.; Garcia, Ernesto; Garcia, Jose L.; Garcia, Pedro (Department of Molecular Microbiology, Centro de Investigaciones Biologicas, CSIC, Madrid, 28006, Spain). Research in Microbiology, 151(6), 437-443 (English) 2000. CODEN: RMCREW. ISSN: 0923-2508. Publisher: Editions Scientifiques et Medicales Elsevier.

AB A review with 27 refs. We have found two **murein hydrolases** (LytB and LytC) tightly bound to the cell envelope that have completely changed the domain building plan previously reported for the **murein hydrolases** of Streptococcus pneumoniae. The active center of LytB and LytC is located at the C-terminal, whereas the binding domain is at the N-terminal. LytC has been characterized as the first lysozyme of S. pneumoniae and behaves as an autolysin at 30°C. LytB appears as the main hydrolase responsible for cell separation since inactivation of lytB leads to the formation of long chains of more than 100 cells. These findings indicate that genetic adaptation of mobile domains is extremely efficient in pneumococcus.

L11 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

2000:211826 Document No. 133:130576 The Staphylococcus aureus lrgAB operon modulates **murein hydrolase** activity and penicillin tolerance. Groicher, Kajetan H.; Firek, Brian A.; Fujimoto, David F.; Bayles, Kenneth W. (Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID, 83844-3052, USA). Journal of Bacteriology, 182(7), 1794-1801 (English) 2000. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB Previous studies in our laboratory have shown that the Staphylococcus aureus LytSR two-component regulatory system affects **murein hydrolase** activity and **autolysis**. A LytSR-regulated dicistronic operon has also been identified and shown to encode two potential membrane-associated proteins, designated LrgA and LrgB, hypothesized to be involved in the control of **murein hydrolase** activity. In the present study, a lrgAB mutant strain was generated and analyzed to test this hypothesis. Zymog. and quant. anal. of **murein hydrolase** activity revealed that the lrgAB mutant produced increased extracellular **murein hydrolase** activity compared to that of the wild-type strain. Complementation of the lrgAB defect by providing the lrgAB genes in trans restored the wild-type phenotype, indicating that these genes confer neg. control on extracellular **murein hydrolase** activity. In addition to these effects, the influence of the lrgAB mutation on penicillin-induced lysis and killing was examined These studies demonstrated that the lrgAB mutation enhanced penicillin-induced killing of cells approaching the stationary phase of growth, the time at which the lrgAB operon was shown to be maximally expressed. This effect of the lrgAB mutation on penicillin-induced killing was shown to be independent of cell lysis. In contrast, the lrgAB mutation did not affect penicillin-induced killing of cells growing in early-exponential phase, a time in which lrgAB expression was shown to be minimal. However, expression of the lrgAB operon in early-exponential-phase cells inhibited penicillin-induced killing, again independent of cell lysis. The data generated by this study suggest that penicillin-induced killing of S. aureus involves a novel regulator of **murein hydrolase** activity.

L11 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1998:463375 Document No. 129:186580 Opposing roles of the Staphylococcus aureus virulence regulators, Agr and Sar, in Triton X-100- and penicillin-induced **autolysis**. Fujimoto, David F.; Bayles, Kenneth W. (Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, ID, 83844-3052, USA). Journal of Bacteriology, 180(14), 3724-3726 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The regulation of **murein hydrolases** is a critical aspect of peptidoglycan growth and metabolism In the present study, we demonstrate that mutations within the Staphylococcus aureus virulence factor regulatory genes, agr and sar, affect **autolysis**, resulting in decreased and increased **autolysis** rates, resp. Zymog. analyses of these mutant strains suggest that agr and sar exert

their effects on **autolysis**, in part, by modulating **murein hydrolase** expression and/or activity.

L11 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1997:386026 Document No. 127:77673 **Murein hydrolases**: a novel target for novel antibiotics. Holtje, Joachim-Volker (Max-Planck-Institut fur Entwicklungsbiologie, Abteilung Biochemie, Tubingen, D-77076, Germany). BIOspektrum (Sonderausg.), 63-66 (English) 1997. CODEN: BOSPFD. ISSN: 0947-0867. Publisher: Spektrum Akademischer Verlag.

AB A review with 25 refs. The **murein hydrolases** that are functioning as pacemaker enzymes of murein growth and thereby of cell propagation immediately turn into suicidal autolytic enzymes as soon as the cellular control circuits are interrupted. Consequently, both activators as well as inhibitors of **murein hydrolases** are likely to affect bacterial growth dramatically. Inhibitors may block further growth by interfering with the pacemaker function, whereas activators may cause **autolysis** from within due to uncontrolled cleavage of murein net. The activity of the **murein hydrolases** could either be affected by a direct or indirectly by interfering with the control mechanisms.

L11 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1996:722752 Document No. 126:44754 Role of precursor translocation in coordination of murein and phospholipid synthesis in Escherichia coli. Ehlert, Kerstin; Volker Hoeltje, Joachim (Abteilung Biol., Max-Planck-Inst. Entwicklungsbiol., Tuebingen, 72076, Germany). Journal of Bacteriology, 178(23), 6766-6771 (English) 1996. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB Inhibition of phospholipid synthesis in Escherichia coli by either cerulenin treatment or glycerol starvation of a glycerol-auxotrophic mutant resulted in a concomitant block of murein synthesis. The intracellular pool of cytoplasmic and lipid-linked murein precursors was not affected by an inhibition of phospholipid synthesis, nor was the activity of the penicillin-binding proteins. In addition, a decrease in the activity of the two lipoprotein **murein hydrolases**, the lytic transglycosylases A and B, could not be demonstrated. The indirect inhibition of murein synthesis by cerulenin resulted in a 68% decrease of trimeric muropeptide structures, proposed to represent the attachment points of newly added murein. Importantly, inhibition of phospholipid synthesis also inhibited O-antigen synthesis with a sensitivity and kinetics similar to those of murein synthesis. It is concluded that the step common for murein and O-antigen synthesis, the translocation of the resp. bactoprenolphosphate-linked precursor mols., is affected by an inhibition of phospholipid synthesis. Consistent with this assumption, it was shown that murein synthesis no longer depends on ongoing phospholipid synthesis in ether-permeabilized cells. We propose that the assembly of a murein-synthesizing machinery, a multienzyme complex consisting of **murein hydrolases** and synthases, at specific sites of the membrane, where integral membrane proteins such as RodA and FtsW facilitate the translocation of the lipid-linked murein precursors to the periplasm, depends on ongoing phospholipid synthesis. This would explain the well-known phenomenon that both murein synthesis and antibiotic-induced **autolysis** depend on phospholipid synthesis and thereby indirectly on the stringent control.

L11 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1996:75082 Document No. 124:137385 Identification and molecular characterization of a putative regulatory locus that affects **autolysis** in Staphylococcus aureus. Brunskill, Eric W.; Bayles,

Kenneth W. (Program Mol. Cell Biol., Univ. Maryland, Baltimore, MD, 21228, USA). Journal of Bacteriology, 178(3), 611-18 (English) 1996. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB Previously in our laboratory, a PCR-based strategy was used to isolate potential sensor gene fragments from the Staphylococcus aureus genome. One DNA fragment was isolated that shared strong sequence similarity to genes encoding bacterial sensor proteins, indicating that it originated from within a potential staphylococcal sensor protein gene. In this study, the DNA surrounding the PCR product origin was cloned and sequenced. This anal. revealed the presence of two genes, termed lytS and lytR, whose deduced amino acid sequences were similar to those of members of the two-component regulatory system family of proteins. S. aureus cells containing an insertional disruption of lytS exhibited a marked propensity to form aggregates in liquid culture, suggesting that alterations in cell surface components exist in this strain. Transmission electron microscopic examination of these cells revealed that the cell surface was rough and diffuse and that a large proportion of the cell population had lysed. The lytS mutant also exhibited increased **autolysis** and an altered level of **murein hydrolase** activity produced compared with the parental strain, NCTC 8325-4. These data suggest that the lytS and lytR gene products control the rate of **autolysis** in S. aureus by affecting the intrinsic **murein hydrolase** activity associated with the cell.

L11 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1995:952867 Document No. 124:255288 From growth to **autolysis**: the **murein hydrolases** in Escherichia coli. Hoeltje, Joachim-Volker (Abteilung Biochemie, Max-Planck-Institut Entwicklungsbiologie, Tuebingen, D-72076, Germany). Archives of Microbiology, 164(4), 243-54 (English) 1995. CODEN: AMICCW. ISSN: 0302-8933. Publisher: Springer.

AB A review with 95 refs. including the **murein hydrolases** of E. coli, their function during bacterial growth and division, their regulation and control.

L11 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1993:533900 Document No. 119:133900 Peptidoglycan (**murein**) **hydrolases**: unusual enzymes for unusual substrates. Schockman, Gerald D.; Chu, Chien Peng; Kariyama, Reiko; Tepper, Lori K.; Daneo-Moore, Lolita (Sch. Med., Temple Univ., Philadelphia, PA, 19140, USA). FEMS Symposium, 65(Bacterial Growth and Lysis), 213-27 (English) 1993. CODEN: FEMSDW. ISSN: 0163-9188.

AB A review, with many refs., on bacteria enzymes that hydrolyze peptidoglycans of the cell wall, causing **autolysis**. Emphasis is given to Enterococcus hirae muramidase-1 and -2.

L11 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1992:190759 Document No. 116:190759 Failure to trigger the autolytic enzymes in minicells of Escherichia coli. Markiewicz, Zdislaw; Hoeltje, Joachim Volker (Abt. Biochem., Max-Planck-Inst. Entwicklungsbiol., Tuebingen, 7400, Germany). FEMS Microbiology Letters, 91(2), 119-23 (English) 1992. CODEN: FMLED7. ISSN: 0378-1097.

AB Minicells from E. coli P678-54 are refractory towards procedures known to induce bacteriolysis of DNA-containing E. coli cells. Although still engaged in murein synthesis, minicells could not be lysed by penicillin G. Likewise, endogenous overprodn. of the cloned soluble lytic transglycosylase, the predominant murein hydrolytic activity in E. coli, failed to lyse minicells. Induction of the phage MS2 lysis protein, a hydrophobic protein assumed to trigger the autolytic system of the host, did not result in bacteriolysis. It

is concluded that the **murein hydrolases** present in minicells are under a tight cellular control.

L11 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1990:420674 Document No. 113:20674 Control of the activity of the soluble lytic transglycosylase by the stringent response in Escherichia coli. Betzner, A. S.; Ferreira, L. C. S.; Hoeltje, J. V.; Keck, W. (Abt. Biochem., Max-Planck-Inst. Entwicklungsbiol., Tuebingen, 7400, Germany). FEMS Microbiology Letters, 67(1-2), 161-4 (English) 1990. CODEN: FMLED7. ISSN: 0378-1097.

AB The soluble lytic transglycosylase (Slt) of E. coli is known to be a powerful **murein hydrolase** in vitro. It is shown here to act as an autolysin in vivo as well. Rapid **autolysis** of Slt overproducing cells was induced by protein biosynthesis inhibitors, which also block the formation of ppGpp. When amino acid starvation was used to inhibit protein synthesis, **autolysis** was suppressed in relA+ but not in relA- cells. These findings indicate that the stringent control modulates the enzymic activity of the Slt in vivo.

L11 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1986:568748 Document No. 105:168748 Phenotypic correction of Streptococcus pneumoniae treated with amidase induced by bacteriophage Dp-1. Garcia, Pedro; Garcia, Ernesto; Ronda, Concepcion; Lopez, Rubens (Inst. Inmunol. Biol. Microbiana, Madrid, 28006, Spain). Microbiologia (Madrid), 1(1-2), 35-41 (Spanish) 1985. CODEN: MICBE3. ISSN: 0213-4101.

AB A phage-associated **murein hydrolase** activity (PAL) induced in an **autolysis**-defective mutant of S. pneumoniae infected with the bacteriophage Dp-1 has been isolated, purified to electrophoretic homogeneity, and biochem. characterized as an endo-N-acetyl-muramyl-L-alanine amidase. The PAL and the inactive form of the host cell autolysin show a marked biochem. similarity, although they differ in their immunol. characteristics. The PAL was adsorbed onto a live, defective mutant of pneumococcus (cwl) and such cells reverted to the wild type phenotype (cured cells) in some important characteristics, such as lysis of the culture in the stationary phase, protoplast formation by hypertonic sucrose, and bacteriolytic response to penicillin, in contrast with the bacteriostatic response of the non-cured cwl. The adsorbed enzyme segregates during growth of the cured cells. PAL acts in the phenotypically cured cells in a similar way to that previously described for the host enzyme. The finding that the autolysins play a direct role in the irreversible effects produced in S. pneumoniae by the β-lactam antibiotics was confirmed.

L11 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1985:556950 Document No. 103:156950 **Murein hydrolases** of Caulobacter crescentus. Markiewicz, Zdzislaw (Inst. Microbiol., Univ. Warsaw, Warsaw, 00-046, Pol.). Acta Microbiologica Polonica, 34(2), 121-9 (English) 1985. CODEN: AMPOAX. ISSN: 0001-6195.

AB C. crescentus Exhibited a similar autolytic response to a variety of factors affecting the structure of the cell envelope and interfering with murein synthesis as several other species of bacteria. **Autolysis** was accompanied by the hydrolysis of murein with the release of soluble degradation products. Several **murein hydrolases** with different bond specificities were found, and, except for the absence of DD-carboxypeptidase and LD-carboxypeptidase activities, the make-up of these enzymes resembled that of Escherichia coli.

L11 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN
1985:501884 Document No. 103:101884 Competence-specific **autolysis** in Streptococcus sanguis. Horne, Diane; Tomasz, Alexander (Rockefeller

Univ., New York, NY, USA). Journal of General Microbiology, 131(3), 533-41 (English) 1985. CODEN: JGMIAN. ISSN: 0022-1287.

AB S. sanguis Strain Wicky activated to competence for genetic transformation is known to undergo a rapid decrease in optical d. upon transfer to an alkaline buffer containing reducing agents. This **autolysis**-like process was specific because preincubation of the competence inducing factor with a specific inactivating protein prevented both cellular lysis and acquisition of competence for genetic transformation. The optical d. decrease of competent bacteria involved the release of a large fraction of intracellular protein, RNA, and lipid. However, no hydrolysis of phospholipid and no degradation of cell wall polymers, including peptidoglycan, could be detected. No peptidoglycan hydrolase activity capable of degrading radiolabeled S. sanguis cell walls was detected in unfractionated S. sanguis exts. It is suggested that **autolysis** of competent S. sanguis involves the activity of a novel type of **murein hydrolase** that introduces only a limited number of bond breaks into the peptidoglycan.

L11 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1983:139567 Document No. 98:139567 A phage-associated **murein hydrolase** in Streptococcus pneumoniae infected with bacteriophage Dp-1. Garcia, Pedro; Garcia, Ernesto; Ronda, Concepcion; Lopez, Rubens; Tomasz, Alexander (Inst. Immunol. Biol. Microbiana, CSIC, Madrid, Spain). Journal of General Microbiology, 129(2), 489-97 (English) 1983. CODEN: JGMIAN. ISSN: 0022-1287.

AB A phage-associated **murein hydrolase** activity capable of degrading pneumococcal cell walls was isolated and purified to homogeneity from the phage-induced lysate of an **autolysis**-defective pneumococcal mutant infected with the bacteriophage Dp-1. Some properties of the enzyme resembled those of the wild-type (host) pneumococcal **murein hydrolase**: cell walls prepared from ethanolamine-grown pneumococci were resistant to the enzyme and the activity was inhibited by the Forssman antigen and was sensitive to proteolytic enzymes. The phage-associated enzyme was not inhibited by antiserum prepared against the purified pneumococcal **murein hydrolase**. The activity was stimulated by reducing agents and was partially inhibited by cardiolipin. The subunit mol. weight of the phage-associated enzyme was somewhat smaller (31,000) than that of the pneumococcal hydrolase (35,000). This appears to be the 1st description of a phage-associated murine hydrolase activity in pneumococci.

L11 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2004 ACS on STN

1982:118562 Document No. 96:118562 **Autolysis** of a division mutant of Escherichia coli. Karibian, Doris; Pellon, G.; Starka, J. (Lab. Physiol. MIcrobienne, Univ. d'Aix-Marseille, Marseille, 13009, Fr.). Journal of General Microbiology, 126(1), 55-61 (English) 1981. CODEN: JGMIAN. ISSN: 0022-1287.

AB The pleiotropic character of the envC chain-forming mutant of E. coli includes leakage of periplasmic enzymes and an abnormal tendency to autolyze. Washed suspensions of envC cells released murein fragments into the supernatant, and cell exts. from the mutant were richer than those of the wild type in exo-β-N-acetylglucosaminidase (187% of the wild-type value) and in soluble endopeptidase (256%) activities, but N-acetylmuramoylamidase, D,D-carboxypeptidase, L,D-carboxypeptidase, and transglycosylase were not markedly different. When envC cells were grown in medium containing 0.58M sucrose, the chains broke up into rods, the L,D-carboxypeptidase activity increased .apprx.6-fold, and D,D-carboxypeptidase 1B .apprx.2-fold. Thus, L,D-carboxypeptidase may be involved in septum splitting. The triggering of **autolysis** in E. coli envC probably depends on the alteration of envelope constituents rather than on an enhanced activity of **murein hydrolases** .

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=> E CHEUNG A/AU
=> S E3,E56-E58
             30 "CHEUNG A"/AU
             18 "CHEUNG AMBROSE"/AU
              1 "CHEUNG AMBROSE I"/AU
             61 "CHEUNG AMBROSE L"/AU
L12         110 ("CHEUNG A"/AU OR "CHEUNG AMBROSE"/AU OR "CHEUNG AMBROSE I"/AU
                OR "CHEUNG AMBROSE L"/AU)

=> S L12 AND L7
L13           4 L12 AND L7

=> S L13 NOT (L5,L9,L11)
L14           1 L13 NOT ((L5 OR L9 OR L11))

=> D CBIB ABS

L14  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2004 ACS on STN
2003:511951   Document No. 139:79117  Sequences of Staphylococcus aureus
      protein Rat and use for identifying agents which regulate autolytic
      processes in bacteria.  Cheung, Ambrose (USA).  U.S. Pat. Appl.
      Publ. US 2003124597 A1 20030703, 20 pp., Cont.-in-part of U.S. Ser. No.
      92,264.  (English).  CODEN: USXXCO.  APPLICATION: US 2002-290143 20021106.
      PRIORITY: US 2001-PV273791 20010306; US 2001-PV312546 20010815; US
      2001-PV329140 20011012; US 2002-92264 20020306.
AB    The invention provides DNA sequence of a novel Staphylococcus aureus protein
      Rat which regulates the autolytic activity.  Methods for identifying and using
      agents which interact with the gene to inhibit bacterial growth and
      infectivity also are provided.
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